

IN THE CLAIMS:

1.-83. (cancelled)

84. (currently amended) A method for amplifying a target nucleic acid sequence comprising a target nucleic acid, the method comprising the steps of:

a) hybridizing a riboprimers to a DNA template comprising the target nucleic acid sequence, wherein said riboprimers comprises: i) only ribonucleotides, or ii) only purine ribonucleotides and only pyrimidine nucleotides, wherein at least one of the pyrimidine nucleotides is a pyrimidine 2'-deoxyribonucleotide having a non-canonical substituent, which substituent is neither an H nor an OH, on the 2'-position of the deoxyribose sugar moiety;

b) extending the riboprimers with a DNA polymerase that lacks 5'-to-3' exonuclease activity; and

c) cleaving the annealed riboprimers with an RNase H enzyme such that another riboprimers hybridizes to the template and repeats primer extension, whereby multiple copies of the complementary sequence of the target nucleic acid sequence are produced.

85. (previously presented) The method of claim 84, further comprising, prior to step (b), the step of hybridizing a blocking oligonucleotide to a region of the template that is 5' with respect to hybridization of the riboprimers to the template.

86. (previously presented) The method of claim 84, wherein the method is conducted under isothermal conditions.

87. (previously presented) The method of claim 84, further comprising the step of:

c) attaching the multiple copies produced in step c) onto a solid substrate to make a microarray of the multiple copies.

88. (previously presented) The method of claim 84, further comprising the step of hybridizing the multiple copies produced in step c) to a microarray of nucleic acid molecules immobilized on a surface of a solid phase.

89. (previously presented) The method of claim 84, wherein step (b) comprises utilization of at least one type of labeled dNTP such that labeled extension products are generated.

90. (previously presented) The method of claim 84, wherein the riboprimers comprises AMP, GMP, 2'-F-dUMP, and 2'-F-dCMP.

91. (previously presented) The method of claim 84, wherein a plurality of riboprimers is used.

92. (previously presented) The method of claim 84, wherein the riboprimers comprises only ribonucleotides.

93. (previously presented) The method of claim 84, wherein the 2'-substituent is selected from the group consisting of a fluorine substituent, an amino substituent, a methoxy substituent, and an azido substituent.

94. (previously presented) The method of claim 84, wherein the riboprimers comprises purine ribonucleotides and pyrimidine 2'-fluoro-2'-deoxyribonucleotides.

95. (previously presented) The method of claim 84, wherein the entire sequence of the riboprimers is complementary to the 3'-end portion of the target sequence.

96. (previously presented) The method of claim 84, wherein the 5'-end portion of the riboprimers is not complementary to the target nucleic acid sequence.

97. (previously presented) The method of claim 96, wherein the non-complementary 5' end portion provides a sequence that can be copied by second-strand primer extension using as a template a first-strand cDNA primer extension product made using the riboprimer.

98. (previously presented) The method of claim 96 wherein the target-complementary sequence at the 3'-end portion of the riboprimer comprises a randomized sequence.

99. (previously presented) The method of claim 85, wherein the blocking oligo comprises a peptide nucleic acid (PNA).

100. (previously presented) The method of claim 84, wherein the DNA polymerase is selected from the group consisting of BST DNA polymerase large fragment (ISOTHERM), SEQUITHERM DNA polymerase (EPICENTRE Technologies), BCABEST DNA polymerase (Takara Shuzo Co.), phi29 DNA polymerase, and Exo-minus Klenow DNA polymerase.

101. (previously presented) The method of claim 84, wherein the RNase H enzyme is a thermostable RNase H.

102. (previously presented) The method of claim 101, wherein said thermostable RNase H is selected from the group consisting of HYBRIDASE thermostable RNase H (EPICENTRE Technologies), Tth RNase H, and Tfl RNase H.

103. (previously presented) The method of claim 84, wherein said RNase H is *E. coli* RNase H.

104. (currently amended) A method for amplifying a target nucleic acid sequence comprising a target nucleic acid, the method comprising:

- a) obtaining a DNA comprising a target nucleic acid sequence;
- b) obtaining a riboprimers, the riboprimers comprising: i) only ribonucleotides, or ii) only purine ribonucleotides and only pyrimidine nucleotides, wherein at least one of the pyrimidine nucleotides is a pyrimidine 2'-deoxyribonucleotide having a non-canonical substituent, which substituent is neither an H nor an OH, on the 2'-position of the deoxyribose sugar moiety, and wherein at least the 3'-end portion of the riboprimers is complementary to a portion of the target nucleic acid sequence;
- c) annealing the riboprimers to the DNA;
- d) obtaining a strand-displacing DNA polymerase that lacks 5'-to-3' exonuclease activity;
- e) primers extending the riboprimers annealed to the DNA with the strand-displacing DNA polymerase under strand-displacing polymerization conditions;
- f) obtaining a double-stranded complex comprising the DNA and a primer extension product, wherein the primer extension product comprises the riboprimers sequence in its 5'-end portion and the target sequence in its 3'-end portion;
- g) contacting the double-stranded complex with an RNase H enzyme under enzyme reaction conditions so as to release at least a portion of the riboprimers sequence in the 5'-end portion of the primer extension product of the double-stranded complex;
- h) annealing a second riboprimers to the single-stranded DNA of the double-stranded complex, wherein the second riboprimers anneals to the single-stranded DNA at the position where the portion of the riboprimers sequence of the primer extension product was released;
- i) primers extending the second riboprimers annealed to the single-stranded DNA of the double-stranded complex with the strand-displacing DNA polymerase under strand-displacing polymerization conditions, so as to displace the first primer extension product from the double-stranded complex and obtain a second double-stranded complex comprising the single-stranded DNA and a second primer extension product; and

- j) obtaining the primer extension product that was displaced from the double-stranded complex as a result of extending the second riboprimers annealed to the single-stranded DNA.

105. (previously presented) The method of claim 104, further comprising the step of annealing a blocking oligo to a region of the DNA, wherein the 5'-end of the blocking oligo that is annealed to the single-stranded DNA delimits the 3'-end of the target nucleic acid sequence.

106. (previously presented) The method of claim 104, further comprising step k) repeating steps b through j whereby multiple copies of the primer extension products corresponding to the target sequence are produced.

107. (previously presented) The method of claim 104 further comprising step l) detecting the primer extension products produced.

108. (previously presented) The method of claim 104, further comprising step l) quantifying the primer extension products.

109. (previously presented) The method of claim 104, wherein a plurality of riboprimers is used.

110. (previously presented) The method of claim 104, wherein the riboprimers comprises only ribonucleotides.

111. (previously presented) The method of claim 104, wherein the 2'-substituent is selected from the group consisting of a fluorine substituent, an amino substituent, a methoxy substituent, and an azido substituent.

112. (previously presented) The method of claim 104, wherein the riboprimers comprises purine ribonucleotides and pyrimidine 2'-fluoro-2'-deoxyribonucleotides.

113. (previously presented) The method of claim 104, wherein the entire sequence of the riboprimers is complementary to the 3'-end portion of the target sequence.

114. (previously presented) The method of claim 104, wherein the 5'-end portion of the riboprimers is not complementary to the target nucleic acid sequence.

115. (previously presented) The method of claim 114, wherein the non-complementary 5' end portion provides a sequence that can be copied by second-strand primer extension using as a template a first-strand cDNA primer extension product made using the riboprimers.

116. (previously presented) The method of claim 114, wherein the target-complementary sequence at the 3'-end portion of the riboprimers comprises a randomized sequence.

117. (previously presented) The method of claim 105, wherein the blocking oligo comprises a peptide nucleic acid (PNA).

118. (previously presented) The method of claim 104, wherein the DNA polymerase is selected from the group consisting of BST DNA polymerase large fragment (ISOTHERM), SEQUITHERM DNA polymerase (EPICENTRE Technologies), BCABEST DNA polymerase (Takara Shuzo Co.), phi29 DNA polymerase, and Exo-minus Klenow DNA polymerase.

119. (previously presented) The method of claim 104, wherein the RNase H enzyme is a thermostable RNase H.

120. (previously presented) The method of claim 119, wherein said thermostable RNase H is selected from the group consisting of HYBRIDASE thermostable RNase H (EPICENTRE Technologies), Tth RNase H, and Tfi RNase H.

121. (previously presented) The method of claim 104, wherein said RNase H is *E. coli* RNase H.

122. (currently amended) A method of generating multiple copies of a polynucleotide sequence complementary to an RNA sequence of interest, the method comprising the steps of:

- a) extending a first primer hybridized to a target RNA with an RNA-dependent DNA polymerase, wherein the first primer is a riboprimer, wherein said riboprimer comprises: i) only ribonucleotides, or ii) only purine ribonucleotides and only pyrimidine nucleotides, wherein at least one of the pyrimidine nucleotides is a pyrimidine 2'-deoxyribonucleotide having a non-canonical substituent, which substituent is neither an H nor an OH, on the 2'-position of the deoxyribose sugar moiety, and whereby a complex comprising a first primer extension product and the target RNA is produced;
- b) cleaving RNA in the complex of step (a) with an RNase H enzyme;
- c) extending a second primer hybridized to the first primer extension product with a DNA-dependent DNA polymerase that lacks 5'-to-3' exonuclease activity, wherein said second primer is a riboprimer comprising: i) only ribonucleotides, or ii) only purine ribonucleotides and only pyrimidine nucleotides, wherein at least one of the pyrimidine nucleotides is a pyrimidine 2'-deoxyribonucleotide having a non-canonical substituent, which substituent is neither an H nor an OH, on the 2'-position of the deoxyribose sugar moiety, and whereby a second primer extension product is produced to form a complex of first and second primer extension products;
- d) cleaving the riboprimer in the complex of first and second primer extension products with an RNase H enzyme such that a riboprimer hybridizes to the second primer extension product; and
- e) extending the riboprimer hybridized to the second primer extension product with a DNA-dependent DNA polymerase,

whereby the first primer extension product is displaced and multiple copies of a polynucleotide sequence complementary to the RNA sequence of interest are generated.

123. (previously presented) The method of claim 122, wherein the RNA-dependent DNA polymerase is selected from the group consisting of Bst DNA polymerase, BST DNA polymerase large fragment (ISOTHERM), Moloney murine leukemia virus (MMLV) reverse

transcriptase, and avian myeloblastosis virus (AMV) reverse transcriptase.

124. (previously presented) The method of claim 123, wherein said MMLV reverse transcriptase comprises RNase H minus MMLV reverse transcriptase.

125. (previously presented) The method of claim 122, wherein a plurality of riboprimers is used to generate multiple copies of a polynucleotide sequence complementary to the RNA sequence of interest.

126. (previously presented) The method of claim 122, wherein a plurality of different riboprimers is used for hybridizing to the target RNA.

127. (previously presented) The method of claim 122, wherein the riboprimers comprises only ribonucleotides.

128. (previously presented) The method of claim 122, wherein the 2'-substituent is selected from the group consisting of a fluorine substituent, an amino substituent, a methoxy substituent, and an azido substituent.

129. (previously presented) The method of claim 122, wherein the riboprimers comprises purine ribonucleotides and pyrimidine 2'-fluoro-2'-deoxyribonucleotides.

130. (previously presented) The method of claim 122, wherein the riboprimers comprises AMP, GMP, 2'-F-dUMP, and 2'-F-dCMP.

131. (previously presented) The method of claim 122, wherein the riboprimers that hybridizes to the target RNA comprises a poly-U sequence.

132. (previously presented) The method of claim 122, wherein the riboprimers that hybridizes to the target RNA is a random primer.

133. (previously presented) The method of claim 122, wherein the riboprimers that hybridizes to the target RNA comprises a 5'-portion that is not hybridizable to the target RNA under conditions under which the riboprimers hybridizes to the target RNA.

134. (previously presented) The method of claim 122, wherein the target RNA is mRNA.

135. (previously presented) The method of claim 122, wherein the RNase H enzyme is a thermostable RNase H.

136. (previously presented) The method of claim 135, wherein said thermostable RNase H is selected from the group consisting of HYBRIDASE thermostable RNase H (EPICENTRE Technologies), Tth RNase H, and Tfi RNase H.

137. (previously presented) The method of claim 122, wherein said RNase H is *E. coli* RNase H.

138. (previously presented) The method of claim 122, wherein the DNA polymerase is selected from the group consisting of BST DNA polymerase large fragment (ISOTHERM), SEQUITHERM DNA polymerase (EPICENTRE Technologies), BCABEST DNA polymerase (Takara Shuzo Co.), phi29 DNA polymerase, and Exo-minus Klenow DNA polymerase.

139. (previously presented) The method of claim 122, wherein the second primer is a random primer.

140. (previously presented) The method of claim 122, wherein the second primer comprises a fragment of the target RNA hybridized to the primer extension product, which fragment is generated by cleaving RNA in the complex in step (b) with an enzyme that cleaves RNA from an RNA/DNA hybrid.

141. (previously presented) The method of claim 122, wherein the second primer comprises DNA.

142. (previously presented) The method of claim 122, wherein the RNA-dependent DNA polymerase and DNA-dependent polymerase are the same enzyme.

143. (previously presented) The method of claim 140 wherein the RNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA hybrid are the same enzyme.

144. (previously presented) The method of claim 122, further comprising the step of:

f) generating multiple copies of a polynucleotide sequence complementary to two or more different sequences of interest.

145. (currently amended) The method of claim 144, wherein at least two different riboprimers that hybridize to the target RNA are used.

146. (previously presented) The method of claim 122, further comprising the step of:

f) attaching the multiple copies onto a solid substrate to make a microarray of the multiple copies.

147. (previously presented) The method of claim 122, further comprising the step of: f)

hybridizing the multiple copies to a microarray of nucleic acid molecules immobilized on a surface of a solid phase.